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This project is being conducted as part of Associated Project #3 of the International Cooperative Biodiversity Group Program under the direction of the Walter Reed Army Institute for Research. The focus of this ICBG Program's study is plants of West and Central Africa. During the reporting period, a total of 41 plant extracts were received from WRAIR, an additional eight compounds were received from the University of Dschang (Cameroon, AP#2). These agents were tested *in vitro* vs. strains of African trypanosomes (*Trypanosoma brucei* group) and of pathogenic trichomonads (*Trichomonas vaginalis* and *Tritrichomonas foetus*). An additional 68 extracts were received from WRAIR in late February and are now entering preliminary screening.

For the trypanosome screens, 39 of 41 WRAIR extracts and all eight of the AP #2 extracts were tested *in vitro* vs. three strains. Eight extracts were tested vs. two strains of *T. vaginalis* and 22 were tested vs. *T. foetus*. For trypanosome studies, 21 of the WRAIR extracts and 4 of 8 of the AP#2 extracts had $IC_{50}$ values $\leq 20$ μg/ml. Three of the extracts tested vs *T. vaginalis* had minimal inhibitory concentrations of 0.3–0.6 mg/ml, while 2 of 22 tested vs. *T. foetus* had similarly high activity. Extracts active vs. trypanosomes were tested in a mouse model infection of *T. brucei* at a dose range of 1–25 mg/kg i.p for three days. None of the 21 extracts tested were curative, however, two prolonged life. Some of the plant genera showing activity *in vitro* were: *Afromomum, Aspilia, Chamaecrista, Cryptolepis, Enantia, Hoslundia, Icacina, Uvaria,* and *Pleiocarpa*

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## I. Introduction

This project is Associated Project #3 of the International Cooperative Biodiversity Group (ICBG) which concerns the activities of plant extracts from Central and West Africa on growth of African trypanosomes and pathogenic trichomonads. The African trypanosomes include *Trypanosoma brucei* (veterinary parasite) and *Trypanosoma rhodesiense* (human and veterinary parasite) and the trichomonads include *Trichomonas vaginalis* (human parasite) and *Tritrichomonas foetus* (veterinary parasite).

The past several years have seen an explosion in the number of cases of African sleeping sickness, from an estimated 25,000 cases/year to $\geq$ 500,000 cases/year (WHO 2001), while human trichomoniasis remains a major STD in the United States and throughout the world (Lossik, 1990). Veterinary diseases caused by both groups of parasites continue to inflect economic tolls on farmers and herdsmen. No new readily available drugs have been developed in > 50 years for African sleeping sickness, while pentamidine and the arsenical melarsoprol remain the mainstays for treatment. There is significant evidence of neurotoxicity and fatalities due to the latter agent (Burri and Keiser, 2002; Keiser et al., 2001; Legros and Evans, 1999). Chemotherapy of human trichomoniasis relies solely on metronidazole, developed in the 1950's, while there is no treatment for veterinary trichomoniasis (Yule et al., 1989). With drugs in common use for so long, it is inevitable that resistance develop (Keiser et al., 2001), thus making it important to search for new, effective chemotherapeutic agents.

This project is being conducted as part of Associated Project #3 of the International Cooperative Biodiversity Group (ICBG) Program under the direction of the Walter Reed Army Institute for Research (WRAIR). The focus of this ICBG Program's study is plants of West and Central Africa. During the reporting period, a total of 41 plant extracts were received from WRAIR, and an additional 8 compounds were received from the University of Dschang (Cameroon, AP #2). These agents were tested *in vitro* vs. strains of African trypanosomes (*Trypanosoma brucei* group) and of pathogenic trichomonads (*Trichomonas vaginalis* and *Tritrichomonas foetus*). An additional 68 extracts were received from WRAIR in late February and these are entering preliminary screening at this time.

For the trypanosome screens, 39 of 41 WRAIR extracts and all 8 of the AP #2 extracts were tested *in vitro* vs. three strains. Eight extracts were tested vs. two strains of *T. vaginalis* and 22 were tested vs. *T. foetus*. For trypanosome studies, 21 of the WRAIR extracts and 4 of 8 of the AP #2 extracts had $IC_{50}$ values $\leq$ 20 μg/ml. Three of the extracts tested vs. *T. vaginalis* had minimal inhibitory concentrations (MIC) of 0.3–0.6 mg/ml, while 2 of 22 tested vs. *T. foetus* had similarly high activity. Extracts active vs. trypanosomes were tested in a mouse model infection of *T. brucei* at a dose range of 1–25 mg/kg i.p for three days. None of the 21 extracts tested were curative, however, two prolonged the life of infected animals. Some of the plant genera showing activity *in vitro* were: *Afromomum, Aspilia, Chamaecrista, Cryptolepis, Enantia, Hoslundia, Icacina,* and *Pleiocarpa*; the two extracts active in the trypanosome model infection were from *Icacina trichanta* and *Uvaria chamae.*

## II. Body

a) African trypanosomes. *In vitro* screens with bloodstream form trypanosomes are set up in 24 well plates using duplicate wells of four extract concentrations each (in HMI medium) plus full-growth controls, as detailed in Bacchi et al. (1998). Initial wide concentration curves were followed by narrow-ranging curves to determine $IC_{50}$ values. Strains of trypanosomes used were: *Trypanosoma brucei*, Lab 110 EATRO (veterinary parasite); *Trypanosoma rhodesiense* KETRI 243 (human isolate), *T. rhodesiense* 243As 10-3 (clone of KETRI 243 highly resistant to melarsoprol and pentamidine).

b) Trichomonads. The method used was the minimal inhibitory concentration (MIC) assay developed by Meingassner et al. (1978). Strains used were *T. vaginalis* C1-NIH (ATTC 30001) and a metronidazole-resistant strain, CDC-085 (ATCC 50143). These are incubated aerobically in 96 well plates with triplicate serial dilutions of each extract, and checked at 24 and 48 h.

c) *In vivo* studies. For African trypanosomes, extracts having $IC_{50}$ values of $\leq 20$ µg/ml were tested in a *T. brucei* Lab 110 EATRO mouse model infection (Bacchi et al., 1990). Mice (10–25 g) were infected with $5 \times 10^5$ trypanosomes and treatment was begun 24 h later. Since all extracts were solubilized in 50% DMSO, extracts were diluted in this solvent to achieve correct concentrations. Mice were dosed once daily for three days, by the intraperitoneal route. Animals surviving > 30 days with no evidence of parasites in blood smears are considered cured.

Results

a) African trypanosomes. *In vitro* screening of plant extracts is conducted using bloodstream forms of three isolates: *Trypanosoma brucei* Lab 110 EATRO (veterinary parasite), *Trypanosoma rhodesiense*, KETRI 243, KETRI 243As10-3 (human pathogens). The latter strain is highly melarsoprol resistant and has moderate resistance to pentamidine (Bacchi et al., 1990). The culture methods have been described (Bacchi et al., 1998), and involve culture in 24 well plates (37°C, 5% $CO_2$) using the medium of Hirumi et al. (1989). Plant extracts are dissolved in 100% DMSO (50 mg/ml stock) and diluted with medium. Trypanosomes are cultured for 72 h. in the presence of extract, with one-half the volume of each well replaced daily with fresh medium + plant extracts. Cell counts are made (Coulter Counter, Model Z1) at 48 and 72 h, with data from 48 h. generally used. Control cell counts were $5 \times 10^6$/ml at 48 h. Initially a broad concentration curve is used, followed by a close-ranging curve to establish $IC_{50}$ values. All curves are done in duplicate, with the results expressed as $IC_{50}$ (µg extract/ml medium).

A total of 39 out of 41 extracts received from Dr. Chris Okunji of WRAIR on March 27, 2000 (Table 1), and 8 received from Drs Appollonaire Tsopmo and Pierre Tane of the University of Dschang (Cameroon - AP #2) were tested. Two of the WRAIR extracts were insoluble in 100% DMSO and were not tested. The 39 WRAIR extracts were tested vs. the three trypanosome isolates; 19 of 39 isolates tested had $IC_{50}$ values of $\leq 20$ µg/ml, with 4 having values of $\leq 1$ µg/ml (Table 2). Of the AP #2 (Cameroon) extracts 5 of 8 had $IC_{50}$ values of $\leq 20$ µg/ml (Table 3).

We tested 16 of the most active WRAIR extracts in a mouse model infection using *T. brucei* Lab 110 EATRO (Bacchi et al., 1998). Mice (25–30 g) were infected with $5 \times 10^5$ trypanosomes and the infection allowed to develop for 24 h. Plant extracts were diluted to 5 mg/ ml with 50%

DMSO. Mice (groups of 3) were injected i.p. with 1, 5, 10 or 25 mg/kg extract once daily for three days. Doses higher than 25 mg/kg were not possible, since they would increase the volume of DMSO to a toxic level. In each experiment, three mice served as infected, untreated controls. Control mice died at four days, with the life-span of treated mice compared to that of controls. None of these extracts were curative, although #1876 (*Icacina trichanta*, aq.) and #1891 (*Uvaria chamae*, $CH_2 Cl_2$) extended the life-span of mice 2–3 days in animals receiving 10 or 25 mg/kg (Table 4). Extract #1889 (*Uvaria chamae*, $CH_2 Cl_2$) was highly toxic, with animals receiving 5 mg/kg dose dying within 24 h . of the initial dose. It would be valuable to further purify #1876 and #1891 to find a more active component. Extract #1891 is especially interesting since a separate $CH_2 Cl_2$ extract of *U. chamae* proved highly toxic (#1889) at the same dose levels. Methanol extracts of *U. chamae* (#1890) proved less effective *in vitro* than #1889 and 1891 and were inactive *in vivo*, as was aqueous an extract (#1898).

In late February 2002, WRAIR sent an additional 68 new plant extracts, containing 25 mg/ sample. These are #s SU 2140–2181, 2192, 2194–2220. Screening studies have begun on SU 2140–2170, with initial growth curve screens done vs. the *T. brucei* isolate (Table 5). Eleven extracts gave $IC_{50}$ values < 10 μg/ml: SU 2140, *Premna guadrifolia;* 2145, *Cassia siama*; 2148, *Guarea thompsonii;* 2156, *Goyania long pelara;* 2157, *Culcasis scanders*; 2158–9, *Hyptis suaveolens*; 2162, *Cassytha filiformis;* 2164–65, *Holarrhena floribunda;* 2166, *Jatropha curcas.*

b) <u>Pathogenic trichomonads</u>. *In vitro* screening of extracts was done using three isolates: *Trichomonas vaginalis* ATCC 3001 (metronidazole sensitive); *T. vaginalis* ATCC 50143 (metronidazole resistant); *Tritrichomonas foetus* KV1. The growth assessment method used was the minimal inhibitory concentration (MIC) assay of Mengassner et al. (1978). The organisms are grown in 96 well microliter plates with 200 μl of medium/well. Drug dilutions are made serially (duplicate sets) to give final concentrations of 400–0.78 mg/ml. After 48 h. plates are read by microscope and each well is scored on the basis of motility and growth, with a 4 representing full growth and 0 being no growth and total loss of motility. The lowest concentration producing a 0 in duplicate wells is the MIC.

A total of eight extracts were tested against metronidazole-sensitive (ATCC 30001) and -resistant (ATCC 50143) strains of *T. vaginalis* (Table 6). Two extracts (#1863 and #1868) had MIC values $\leq$ 0.6 mg/ml for the sensitive isolate and four had values of $\leq$ 0.6 mg/ml for the resistant isolate. The active extracts were from *Aspilia africana* (#1863, $CH_3 Cl_2$), *Combretum dulchipetalum* (#1868, Me OH; #1870, aq.), *Enantia chlorontha* (#1873, Me OH), *Mormodica charanta*, $CH_2Cl_2$). In particular, the aqueous extract of *Combretum* (#1870) was highly effective vs. the metronidazole-resistant isolate, at a concentration equal to that of metronidazole (0.3 mg/ml vs. 0.4 mg/ml, respectively, Table 6).

A total of 31 extracts were tested vs. *Tritrichomonas foetus* (Table 7). Of these, four (#1874, 1879, 1895, 1896) had MIC values of 0.6 mg/ml. These were from *Hoslundia opposita* ($CH_2Cl_2$), *Phyllanthus amerus* (aq.) and *Cleistopholis patens* (Me OH, $CH_2Cl_2$), and differed in origin from extracts found active vs. *T. vaginalis*.

In all, eight extracts of 31 tested were active vs. the trichomonad group (Table 8). Of these, four were also active vs. African trypanosomes, indicating that this overlap activity may be a function of generalized cytotoxicity.

c) Training. At Haskins Laboratories of Pace University, the function of our group, in part, is to train undergraduates. This past reporting period, a total of four Pace students worked on various phases of the ICBG project. In addition, an NSF High School Science Teacher Summer Training Institute is held annually at the University (July 23–August 3, 2001). This two week program features the ecosystem as a major topic, and the teachers are given lectures on the meaning of biodiversity including the activities of the ICBG program, and a tour of laboratory facilities. Our group is also working with phytochemists at WRAIR and the University of Dschang towards finalizing several papers for publication.

The only issue of concern is the distribution of plant extracts for use in our sub-project. We would like to see follow-up on certain extracts which seem highly active *in vitro*, for testing *in vivo*, i.e., purification of the active agents in these extracts so that an increase in growth inhibitory activity can be determined, also the possible identification of the chemical nature of the active agent(s).

## III. Key Research Accomplishments

- Identification of 19 WRAIR plant extracts and another 5 from Cameroon (AP #2) out of a total of 47 extracts tested which had $IC_{50}$ values of $\leq$ 20 μg/ml for three African trypanosomes isolates.

- Seven of the WRAIR and 2 of the AP #4 extracts had $IC_{50}$ values of $\leq$ 5 μg/ml for three African trypanosomes isolates.

- Since the purity and the potency of these extracts is not known with respect to the active agents, we believe the most active of these extracts are candidates for further purification and/or *in vivo* testing vs. African trypanosomes.

- Three of eight extracts tested vs. *T. vaginalis* had MIC values $\leq$ 0.6 mg/ml vs. a metronidazole resistant isolate.

- Four of 31 extracts tested vs. *T. foetus* had MIC values of $\leq$ 0.6 mg/ml

- The most active extracts vs. *T. vaginalis* were not those most active against the African trypanosomes, indicating some degree of specificity is present, with fewer of these active vs. trypanosomes having MIC values in the range of 0.3–0.6 mg/ml.

## IV. Reportable Outcomes. None

## V. Conclusions

Recent research has identified a number of plant extracts having significant *in vitro* growth inhibitory activity against African trypanosomes and pathogenic trichomonads. Additional supplies of the extracts are needed to effect extended (5 or 7 day) dosing regimens. Moreover, additional purification of active extracts is needed to allow better control of *in vivo* dosing.

In comparing *in vitro* activities of extracts, it is evident that a degree of specificity was again present – for example, SU-1870 and 1880 were highly effective vs. trypanosomes but not *T. vaginalis* isolates.

Economically, this is an important program for Nigeria and Cameroon: both are endemic for human and veterinary trypanosomiasis, while STD infections, including *T. vaginalis* and bovine trichomoniasis are a significant source of human suffering and an economic drain. There is an urgent need for new and inexpensive drugs for trypanosomiasis. Treatment of human trichomoniasis depends solely on metronidazole, and there is no agent currently available for bovine trichomoniasis [metronidazole kills the microbial flora of the rumen and cannot be given to cattle]. Development of anti-protozoal agents from local plants would be a major factor in the well-being of these populations and a boost to local and national economics.

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**Table 1.** Plant extracts received from WRAIR, March 27, 2000.

| Lab Number | Plant Name | Extract | mg |
|---|---|---|---|
| SU 1863 | *Aspilia africana* | $CH_2Cl_2$ | 50 |
| SU 1864 | *Cassaytha filifoimis* | MeOH | 81 |
| SU 1865 | *Celosia trigyna* | MeOH | 50 |
| SU 1866 | *Chamaecrista mimosoides* | $CH_2Cl_2$ | 55 |
| SU 1867 | *Combretum dulchipetalum* | $CH_2Cl_2$ | 52 |
| SU 1868 | *Combretum dulchipetalum* | MeOH | 50 |
| SU 1869 | *Combretum dulchipetalum* | $CH_2Cl_2$ | 52.7 |
| SU 1870 | *Combretum dulchipetalum* | Aq. | 46 |
| SU 1871 | *Combretum dulchipetalum* | Aq. | 50 |
| SU 1872 | *Cryptolepis sanguinolenta* | $CH_2Cl_2$ | 60 |
| SU 1873 | *Enantia chlorontha* | MeOH | 52.7 |
| SU 1874 | *Hoslundia opposita* | $CH_2Cl_2$ | 50 |
| SU 1875 | *Icacina trichanta* | MeOH | 50 |
| SU 1876 | *Icacina trichanta* | Aq. | 47 |
| SU 1877 | *Mormodica charanta* | $CH_2Cl_2$ | 62 |
| SU 1878 | *Phyllanthus amerus* | $CH_2Cl_2$ | 51 |
| SU 1879 | *Phyllanthus amerus* | Aq. | 60 |
| SU 1880 | *Pleiocarpa pycnantha* | $CH_2Cl_2$ | 60 |
| SU 1881 | *Scoparia dulcis* | $CH_2Cl_2$ | 51 |
| SU 1882 | *Scoparia dulcis* | MeOH | 50 |
| SU 1883 | *Scoparia dulcis* | Aq. | 50 |

**Table 1 (continued)**

| Lab Number | Plant Name | Extract | mg |
|---|---|---|---|
| SU 1884 | *Solenostemon monostachyus* | $CH_2Cl_2$ | 48 |
| SU 1885 | *Solenostemon monostachyus* | Aq. | 50 |
| SU 1886 | *Trimfetta tomentosa* | $CH_2Cl_2$ | 55 |
| SU 1887 | *Trimfetta tomentosa* | MeOH | 50 |
| SU 1888 | *Urena lobata* | $CH_2Cl_2$ | 59 |
| SU 1889 | *Uvaria chamae* | $CH_2Cl_2$ | 59 |
| SU 1890 | *Uvaria chamae* | MeOH | 79 |
| SU 1891 | *Uvaria chamae* | $CH_2Cl_2$ | 89 |
| SU 1892 | *Uvaria chamae* | MeOH | 51 |
| SU 1893 | *Uvaria chamae* | $CH_2Cl_2$ | 58 |
| SU 1894 | *Uvaria chamae* | MeOH | 81 |
| SU 1895 | *Cleistopholis patens* | $CH_2Cl_2$ | 85 |
| SU 1896 | *Cleistopholis patens* | MeOH | 58 |
| SU 1897 | *Crescentia cujete* | $CH_2Cl_2$ | 50 |
| SU 1898 | *Uvaria chamae* | Aq. | 54 |
| SU 1899 | *Uvaria chamae* | Aq. | 50 |
| SU 1900 | BP 16524 | | 40 |
| SU 1901 | BP 16533 | | 40 |
| SU 1902 | *Cleistopholis patens* | Aq. | 52 |
| SU 1903 | *Uvaria chamae* | Aq. | 48 |

**Table 2.** Activity of WRAIR extracts vs. African trypanosomes *in vitro*: *T. brucei* Lab 110 EATRO; *T. rhodesiense* KETRI 243 and 243 As 10-3.

| | $IC_{50}$ (μg/ml) | | |
|---|---|---|---|
| **Extract** | **EATRO 110** | **KETRI 243** | **KETRI 243 As10-3** |
| SU-1863 | 2.4 | 20 | 20.5 |
| SU-1864 | 71 | 225 | 220 |
| SU-1865 | 82 | 140 | 165 |
| SU-1866 | 6.6 | 36 | 25 |
| SU-1867 | 19.5 | 54.5 | 61 |
| SU-1868 | 11 | 16 | 18.5 |
| SU-1869 | 13.5 | 11 | 22 |
| SU-1870 | 7.3 | 6.4 | 6.6 |
| SU-1871 | 18.5 | 17.5 | 22.5 |
| SU-1872 | 0.66 | 5.7 | 4.8 |
| SU-1873 | 6.9 | 9.8 | 8.4 |
| SU-1874 | 5.0 | 1.75 | 28.5 |
| SU-1875 | 7.65 | 5.75 | 6.7 |
| SU-1876 | 22 | 15.5 | 24.5 |
| SU-1877 | 28.5 | 59 | 24.5 |
| SU-1878 | 0.84 | 4.6 | 0.92 |
| SU-1879 | 12.5 | 13.5 | 18.5 |
| SU-1880 | 0.97 | 0.69 | 4.2 |
| SU-1881 | 17.5 | 22.5 | 19.5 |
| SU-1882 | 205 | 215 | 165 |
| SU-1883 | 71 | 200 | 165 |
| SU-1884 | 63.5 | 45.5 | 65 |

**Table 2** (continued)

| Extract | IC50 (µg/ml) | | |
|---|---|---|---|
| | **EATRO 110** | **KETRI 243** | **KETRI 243 As10-3** |
| SU-1885 | 205 | 225 | 180 |
| SU-1886 | 64 | 7.9 | 135 |
| SU-1887 | 129 | 150 | 160 |
| SU-1888 | 145 | 25 | 185 |
| SU-1889 | 3.7 | 36 | 187.5 |
| SU-1890 | 22.5 | 73 | 14.5 |
| SU-1891 | 0.57 | 2.6 | 33.25 |
| SU-1892 | 180 | 190 | 140 |
| SU-1893 | 48 | 21.5 | 74 |
| SU-1894 | 20 | 21.9 | 16.5 |
| SU-1895 | 200 | 64 | 150 |
| SU-1896 | 135 | 175 | |
| SU-1897 | 125 | 295 | 330 |
| SU-1898 | 28.5 | 28.5 | 20.1 |
| SU-1899 | 94 | 190 | 85 |
| SU-1900* | - | - | - |
| SU-1901* | - | - | - |
| SU-1902 | 220 | 225 | 160 |
| SU-1903 | 78 | 175 | 125 |
| Pentamidine | 0.0008 | 0.00098 | 0.00075 |
| Melarsen Oxide | 0.0075 | 0.016 | 0.016 |

*Not soluble in DMSO

**Table 3.** Activity of University of Dschang (AP #2) extracts vs. African trypanosomes *in vitro.*

| | $IC_{50}$ (μg/ml) | | |
|---|---|---|---|
| | | KETRI | |
| | Lab110 EATRO | 243 | 243 As 10-3 |
| ASP | 0.5 | 1.5 | 3.9 |
| $ASS_2$ | 28 | 26.5 | 130 |
| $ASS_4$ | 30.5 | 16 | 51 |
| $ASS_5$ | 55 | 50.1 | 56 |
| $TZM_{1A}$ | 21.5 | 18 | 15.9 |
| $TZM_1$ | 2.35 | 22.5 | 17 |
| $TZM_4$ | 4.45 | 2.1 | 1.85 |
| $TZM_4$ HCl | 3.59 | 3.59 | 1.80 |

**Table 4.** WRAIR extracts with highest activity ($IC_{50} \leq 20$ μg/ml) vs. African trypanosomes and tested *in vivo* in a *T. brucei* model.

| Lab Number | Plant Name | Extract |
|---|---|---|
| *SU 1863 | *Aspilia africana* | $CH_2Cl_2$ |
| SU 1866 | *Chamaecrista mimosoides* | $CH_2Cl_2$ |
| *SU 1870 | *Combretum dulchipetalim* | Aq. |
| SU 1872 | *Cryptolepis sanguinolenta* | $CH_2Cl_2$ |
| *SU 1873 | *Enantia chlorontha* | MeOH |
| *SU 1874 | *Hoslundia opposita* | $CH_2Cl_2$ |
| SU 1875 | *Icacina trichanta* | MeOH |
| SU 1878 | *Phyllanthus amerus* | $CH_2Cl_2$ |
| SU 1880 | *Pleiocarpa pycnantha* | $CH_2Cl_2$ |
| SU 1886 | *Trimfetta tomentosa* | $CH_2Cl_2$ |
| SU 1889 | *Uvaria chamae* | $CH_2Cl_2$ |
| SU 1890 | *Uvaria chamae* | MeOH |
| SU 1891 | *Uvaria chamae* | $CH_2Cl_2$ |
| SU 1894 | *Uvaria chamae* | MeOH |
| SU 1898 | *Uvaria chamae* | aq. |
| SU 1900 | BP 16524 | - |

* Also active vs. trichomonads

**Table 5**. Activity of WRAIR extracts, received February 22, 2002 vs. African trypanosomes *in vitro: T. b. brucei* Lab 110 EATRO.

| **Extract** | **Plant** | **$IC_{50}$ (μg/ml) *T. b. brucei*** |
|---|---|---|
| *SU 2140 | *Premma guadrifolia* | 2.8 |
| SU 2141 | *Asystasis gangetica* | > 100 |
| SU 2142 | *Walthereia indica* (Stems/Leaves) | 25.5 |
| SU 2143 | *Pupalia lappacea* | > 100 |
| SU 2144 | *Grewia cissiodes* (Leaves) | 67 |
| *SU 2145 | *Cassia siamea* (leaves/stems) (dry) | 6.6 |
| SU 2146 | *Uvaria chamae* (Roots) | 28.5 |
| SU 2147 | *Uvaria chamae* (Roots) | > 100 |
| *SU 2148 | *Guarea thompsonli* (Stem bark) | 4.0 |
| SU 2149 | *Guarea thompsonli* (Stem bark) | 12 |
| SU 2150 | *Guarea thompsonli* (Stem bark) | 55 |
| SU 2151 | *Melian excelsa* (Stem bark) | [0.05 - 0.5] |
| SU 2152 | *Solenostemon monostachyus* (Leaves) | [5 - 50] |
| SU 2153 | *Hymenocardia acida* (Leaves) | 27.5 |
| SU 2154 | *Aspienium bulbiferum* (whole plant) | 66 |

*Most active - $IC_{50} < 10$ μg/ml.

**Table 5 (continued)**

| Extract | Plant | $IC_{50}$ (μg/ml) *T. b. brucei* |
|---|---|---|
| SU 2155 | *Asystasia gangetica* | 9.3 |
| *SU 2156 | *Goyania long pelara* (Leaves/stems) | 5.8 |
| *SU 2157 | *Culcasis scanders* (whole plant) | 0.235 |
| *SU 2158 | *Hyptis suaveolens* (leaves) | 0.10 |
| *SU 2159 | *Hyptis suaveolens* (leaves) | 7.5 |
| SU 2160 | *Picralima nitida* (whole plant) | > 100 |
| SU 2161 | *Picralima nitida* (whole plant) | > 100 |
| *SU 2162 | *Cassytha filiformis* (whole plant) | 4.6 |
| SU 2163 | *Cassytha filiformis* (whole plant) | 5.0 |
| *SU 2164 | *Holarrhena floribunda* (leaves) | 0.5 |
| *SU 2165 | *Holarrhena floribunda* (leaves) | 3.8 |
| *SU 2166 | *Jatropha curcas* (leaves) | 5.0 |
| SU 2167 | *Jatropha curcas* (leaves) | [0.05 - 0.5] |
| SU 2168 | *Ritchiea capparoides* (roots) | [0.5 - 5] |
| SU 2169 | *Ritchiea capparoides* (roots) | > 100 |
| SU 2170 | *Hymenocardia acida* (leaves) | [10 - 100] |

*Most active - $IC_{50} < 10$ μg/ml.

**Table 6.** Activity of WRAIR extracts *in vitro* vs. *Trichomonas vaginalis* strains. ATCC 300001 is metronidazole sensitive and ATCC 50143 is resistant. MIC, Minimal Inhibitory Concentration.

| | MIC (mg/ml) | | | |
|---|---|---|---|---|
| | ATCC 30001 | | ATCC 50143 | |
| **Extract** | **24h** | **48h** | **24h** | **48h** |
| SU 1863 | 1.3 | 0.6 | <1.3 | 0.6 |
| SU 1864 | 1.3 | <1.3 | <1.3 | <1.3 |
| SU 1865 | <1.3 | <1.3 | <1.3 | <1.3 |
| SU 1868 | 1.3 | 0.6 | 1.3 | 1.3 |
| SU 1870 | - | - | 0.3 | 0.3 |
| SU 1873 | - | - | 1.3 | 0.6 |
| SU 1876 | - | - | <1.3 | <1.3 |
| SU 1877 | - | - | <1.3 | 0.6 |
| Metronidazole | - | 0.003 | - | 0.40 |

**Table 7.** Activity of WRAIR extracts *in vitro* vs. *T. foetus* KV1.

| Extract | MIC (mg/ml) 24 h | 48 h |
|---|---|---|
| SU 1863 | 1.3 | - |
| SU 1864 | >1.3 | - |
| SU 1865 | >1.3 | - |
| SU 1866 | 1.3 | 1.3 |
| SU 1867 | >1.3 | >1.3 |
| SU 1869 | >1.3 | 1.3 |
| SU 1872 | >1.3 | 1.3 |
| SU 1873 | 1.3 | - |
| SU 1874 | 0.6 | 0.3 |
| SU 1875 | >1.3 | 1.3 |
| SU 1878 | 1.3 | 1.3 |
| SU 1879 | 1.3 | 0.6 |
| SU 1880 | >1.3 | 1.3 |
| SU 1881 | >1.3 | 1.3 |
| SU 1882 | 1.3 | 1.3 |
| SU 1883 | >1.3 | 1.3 |
| SU 1884 | 1.3 | 1.3 |
| SU 1887 | > 1.3 | - |
| SU 1889 | 1.3 | - |
| SU 1890 | > 1.3 | - |
| SU 1891 | >1.3 | 1.3 |
| SU 1892 | >1.3 | >1.3 |
| SU 1893 | 1.3 | 1.3 |
| SU 1894 | >1.3 | >1.3 |
| SU 1895 | 0.63 | 0.63 |
| SU 1896 | 0.31 | - |
| SU 1898 | >1.3 | - |
| SU 1899 | 1.3 | 1.3 |
| SU 1900 | >1.3 | >1.3 |
| SU 1901 | >1.3 | >1.3 |
| SU 1902 | 1.3 | 1.3 |
| Metronidazole | - | 0.003 |

**Table 8.** Most active WRAIR extracts vs. Trichomonads (MIC ≤ 0.6 mg/ml)

| Lab Number | Plant Name | Extract |
|---|---|---|
| *SU 1863 | *Aspilia africana* | $CH_2Cl_2$ |
| SU 1868 | *Combretum dulchipetalim* | MeOH |
| *SU 1870 | *Combretum dulchipetalim* | Aq. |
| *SU 1873 | *Enantia chlorontha* | MeOH |
| *SU 1874 | *Hoslundia opposita* | $CH_2Cl_2$ |
| SU 1877 | *Mormodica charanta* | $CH_2Cl_2$ |
| SU 1879 | *Phyllanthus amerus* | Aq. |
| SU 1895 | *Cleistopholis patent* | $CH_2Cl_2$ |

* Also active vs. trypanosomes